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OBSERVATIONS ON HOSPITALIZED DENGUE PATIENTS
IN MANILA

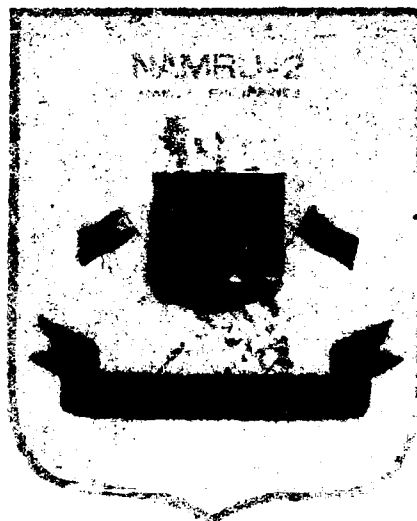
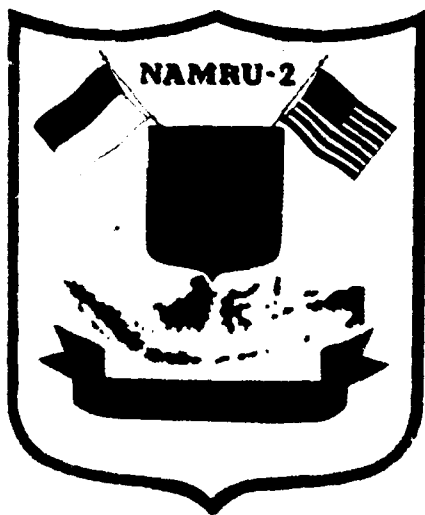
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OBSERVATIONS ON HOSPITALIZED DENGUE PATIENTS IN MANILA

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INTRODUCTION

Dengue hemorrhagic fever (DHF) has been recognized as a major public health problem in the Philippines and parts of southern and southeastern Asia since 1956 when the etiology of the disease was first determined. Observations of the illness among Filipino children were first reported in 1954 when 21 cases were described with a clinical syndrome similar to epidemic hemorrhagic fever in Korea.² In another report two years later, the same authors termed the disease Philippine hemorrhagic fever and described in greater detail the clinical picture of 85 cases.³ In both of these papers, the diagnoses were based on the clinical course of the illness and characteristic laboratory findings.

Detailed clinical, virological and serological studies on DHF were first reported during the 1966 greater Manila epidemic.^{4,5} Since that time, there have only been a few reliable studies published on the various aspects of the disease because of a lack of diagnostic laboratory support.^{6,7}

In 1983, when Manila experienced another outbreak of dengue, the Virology Department of U.S. NAMRU-2 performed viral and serologic tests for the Hospital of Infant Jesus in Manila. This report describes clinical, serological and virological studies of confirmed dengue infections at the hospital.

MATERIALS AND METHODS

Patient selection and clinical studies.

All patients described in this report were admitted

to the Hospital of Infant Jesus between October 31, 1983 and March 31, 1984. Criteria for the diagnosis of dengue infection were based on the hemagglutination-inhibition (HI) test for antibody and on virus isolation. Patients were classified serologically according to their antibody response: (a) primary seroconversion (1°Sc) – if there was a fourfold or greater rise in titer between the acute and another sample taken 1 – 4 weeks later, titer of later sample $\leq 1:640$; (b) secondary seroconversion (2°Sc) – if there was a fourfold or greater rise between the acute and any later sample, the titer of later sample $\geq 1:1280$; (c) presumptive recent secondary (2°PR) – if any single sample had a titer $\geq 1:1280$, no seroconversion; (d) unclassified seroconversion (Uncl. Sc) – if there was a fourfold or greater rise between the acute and a later sample taken less than 7 days later, the titer of later sample $\leq 1:640$. DHF was differentiated from dengue fever (DF), and DHF was classified by disease severity as grades I, II, III and IV according to the guidelines published by the World Health Organization.⁸ Briefly, DHF grade I is characterized by the occurrence of positive tourniquet test as the only hemorrhagic manifestation; whereas, grade II includes spontaneous bleeding as an additional finding. Grades III and IV include findings of circulatory failure and are referred to as dengue shock syndrome. Grade III is defined as narrowing of pulse pressure to $\leq 20\text{mm Hg}$ or hypotension accompanied by cold, clammy skin and restlessness. Grade IV shock occurs when the blood pressure and pulse are undetectable. Grades I and II can be differentiated from cases of DF that develop hemorrhagic manifestations by the presence of concurrent thrombocytopenia ($\leq 100,000/\text{mm}^3$) and hemoconcentration (hematocrit increase by $\geq 20\%$) in the DHF cases.

On admission, patients were carefully observed and pertinent clinical and laboratory data recorded daily on a standard form. These included vital signs, examination for petechiae or other skin hemorrhages, examination for signs of circulatory failure and presence of pleural effusion. X-ray examination of the chest was performed for cases who showed physical findings corresponding to hydrothorax.

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Daily tourniquet tests were performed on all cases by inflating a sphygmomanometer cuff on the arm midway between systolic and diastolic pressure for 8 minutes and then counting the number of petechiae. A count of greater than 20 petechiae/inch² was considered positive.⁸

Hematology

CBC, Hct, and platelet count were performed on most patients using blood samples obtained less than 24 hours after admission. Serial determinations of Hct and platelet counts were done daily or as frequently as every four hours in severely ill patients. The platelet counts were done using the direct method (Rees and Ecker) with Wright's Giemsa stain for the first 6 week weeks of the study; thereafter, the Unopette ® method was used.

Serology

Aliquots of acute blood were tested for dengue (DEN) antibody, and blood samples were drawn for repeat serology upon discharge from the hospital and again approximately two weeks after onset of illness. The sera were tested by the HI test using the method of Clarke and Casals adapted for microtechnique.^{9,10} All sera were tested with DEN 1, DEN 2, DEN 3 and DEN 4 antigens. Paired sera were always tested simultaneously to determine the final diagnostic classification of each patient.

Virus isolation

Virus isolation attempts were done on all acute sera by inoculation onto a mosquito cell line (AP 61).¹¹ Virus isolates were identified as to serotype by the Indirect Fluorescent Antibody Test (IFAT) using type specific DEN monoclonal antibodies.^{12, 13}

Statistical comparisons

The Chi square test with Yates' correction or Fisher's exact test were used to compare differences in the frequency of clinical manifestations between 1^o and 2^o cases.

RESULTS

From October 31, 1983 to March 31, 1984, 379 (43.5%) of 870 clinically suspected cases were confirmed as dengue infections. This represented 7% out of a total of 5,427 admissions at the Hospital of Infant Jesus. The age of the confirmed patients ranged from 8 months to 18 years, and the median age was 6.6 years (Table 1). Although most of the patients (77.6%) were 10 years or below, only 2 of the cases were infants. The male to female ratio was 1:0.97.

The laboratory diagnostic classification of the confirmed cases is shown in Table 2. Most of the patients

(84.9%) showed a 2^o Sc or 2^oPR antibody response. Twenty-four patients who seroconverted and 7 cases from whom virus was isolated from a single acute serum sample could not be classified serologically.

Table 1. Age and sex distribution of 379 patients with confirmed dengue infections

Age range	No. of cases		Total
	Male	Female	
< 1	1	1	2 (0.5)
1 - 3	24	23	47 (12.4)
4 - 6	54	63	177 (30.9)
7 - 10	69	59	128 (33.8)
< 11 - 14	33	30	63 (16.6)
> 15	11	11	22 (5.8)
Total	192	187	379 (100)

Table 2. Classification by laboratory diagnosis of 379 patients with confirmed dengue infections

Antibody response.	No of cases			%
1 ^o Sc	26			(6.9)
2 ^o Sc	124	322		(84.9)
2 ^o PR	198			
Uncl. Sc	24	31		(8.1)
Virus isolation	7*			
				(1.8)

* This number includes only those patients from whom virus was isolated and who could not be classified serologically.

Table 3 shows the frequency of non-specific signs and symptoms. Regardless of the type of antibody response, the most frequent findings were anorexia, skin flushing, vomiting, hepatomegaly and abdominal pain. Pleural effusion and abdominal pain were more frequent in cases with a 2^o type of antibody response ($P = 0.03$ and $P = 0.006$, respectively). Skin rashes occurred more frequently among patients with a 1^o compared to a 2^o antibody response, but the difference was not significant ($P = 0.07$).

Petechiae, positive tourniquet test and epistaxis were the most frequent hemorrhagic manifestations in all cases (Table 4). No significant differences in the frequency of bleeding manifestations were found between 1^o and 2^o antibody responders except for petechiae which were found more frequently in the latter group ($P = .013$). GI bleeding was present in 47 (12.4%) patients, and 40 (85.1%) of these cases had a 2^o antibody response.

Thirty-three patients (8.7%) developed signs and symptoms of circulatory failure, and 31 were patients with a 2^o type of antibody response (Table 4). None of the patients with a 1^o type of antibody response developed

shock. There were 2 fatalities among the shock cases. One fatal case with severe GI bleeding and convulsions had a 2° antibody response. The other fatal case had petechiae, bouts of epistaxis, developed seizures and went into a coma shortly before death. The antibody response could not be

Table 3. Non-specific signs and symptoms observed in 379 patients with confirmed dengue infections

Finding	Serological Classification			Total
	1°	2°**	Other***	
Anorexia	14 (53.8)*	198 (61.4)	18 (58.0)	230 (60.7)
Vomiting	9 (34.6)	146 (45.3)	12 (38.7)	167 (44.1)
Abdominal pain	1 (3.8)	82 (25.4)	7 (22.5)	90 (23.7)
Flushing	13 (50.0)	162 (50.3)	11 (35.4)	186 (49.1)
Rashes	7 (26.9)	45 (13.9)	6 (19.3)	58 (15.3)
Lymph node enlargement	6 (23.1)	39 (19.7)	6 (19.3)	73 (19.3)
Hepatomegaly	9 (34.6)	77 (23.9)	5 (16.1)	91 (24.0)
Pleural effusion	0	41 (12.7)	0	41 (10.8)
Restlessness	1 (3.8)	13 (4.0)	0	14 (3.7)
Convulsion	1 (3.8)	1 (0.3)	0	2 (0.5)
Lethargy	0	2 (0.6)	0	2 (0.5)

* () = %

** Includes 2° Sc and 2° PR cases

*** Includes unclassified SC and cases with (+) virus isolations that could not be classified serologically

Table 4. Hemorrhagic and shock manifestations observed in 379 patients with confirmed dengue infections

Finding	Serological Classification			Total
	1°	2°**	Other***	
+TT	18 (69.2)*	267 (82.9)	28 (90.3)	313 (82.6)
Petechiae	20 (76.9)	299 (92.8)	26 (83.8)	345 (91.0)
Epistaxis	3 (11.5)	48 (14.9)	5 (16.1)	56 (14.8)
Hemoptysis	0	3 (6.9)	1 (3.2)	4 (1.1)
Ecchymosis	0	14 (4.3)	0	14 (3.7)
Gum bleeding	1 (3.8)	6 (1.8)	0	7 (1.8)
Hematuria	2 (7.6)	42 (13.0)	0	44 (11.6)
GI bleeding (Hematemesis/ melena)	3 (11.5)	40 (12.4)	23 (74.1)	47 (12.4)
Shock	0	31 (9.6)****	2 (6.4)****	3 (8.7)

* TT - Tourniquet test

* () = %

** Includes 2° Sc and 2° PR cases

*** Includes unclassified Sc and cases with (+) virus isolations that could not be classified serologically.

**** = 1 fatal case

classified because paired sera were not available; however, DEN 3 was isolated from this patient.

Of the 171 cases that had platelet counts done by the Unopette® method, 96 (56%) had thrombocytopenia, but only two (1%) patients done by the direct method had a platelet count $< 100,000/\text{mm}^3$ (Table 5). Hemoconcentration was observed in 101/361 (27.9%) cases. The majority (57.2%) of the patients had a normal TWBC ($5-10 \times 10^3/\text{mm}^3$). Leucopenia was recorded in 93 cases (25.7%), and leucocytosis in 63 patients (17.5%).

The patients whose platelet counts were done by the Unopette® method were classified clinically according to the WHO criteria (Table 6). Of these 171 cases, only 77 (45%) could be classified as DHF. 7 grade I (4.1%), 56 grade II (32.7%) and 14 grade III (8.2%). The remaining 94 cases (55%) were classified as DF with hemorrhagic manifestations.

Thirty-one strains of dengue were isolated. 12 DEN 1, 6 DEN 2 and 13 DEN 3. Most of the isolates came from patients whose acute sera had an HI antibody titer $\leq 1:20$ (Table 7).

Table 5. Platelet counts in 372 confirmed dengue patients*

Platelet count Range ($\times 10^3/\text{mm}^3$)	Platelet Count Method	
	Unopette	Direct
< 50	21 (12.2)**	0 (0)
50 - 100	75 (43.8)	2 (0.9)
101 - 150	30 (17.5)	16 (7.9)
151 - 200	24 (14.0)	53 (26.3)
201 - 250	11 (6.4)	92 (45.7)
> 250	10 (5.8)	38 (18.9)
Total	171	201

* Different patients were tested by each method.

** () = %

Table 6. Serological responses and WHO clinical classification of 171 confirmed dengue patients whose platelets were counted by the Unopette method

Clinical Classification	No. patients by serological classification			
	1°	2°*	Sc	Total %
DF	10	70	14	94 (55.0)
DHF	0	7	0	77 (45.0)
Grade I	0	7	0	7 (4.1)
Grade II	3	50	3	56 (32.7)
Grade III	0	14	0	14 (8.1)
Grade IV	0	0	0	0 (0)
Total	13	141	17	171

* Includes 2° Sc and 2° PR cases.

Table 7. Distribution of dengue virus isolates by serotype in relation to the homologous HI antibody titers of acute sera from which the viruses were isolated.

Dengue virus serotype	HI Titer			Total
	unknown	<1.20	>1.20	
1	1	10	1	12
2	—	2	4	6
3	4	7	2	13
Total	5	19	7	31

DISCUSSION

Overall, our clinical findings are similar to those reported from other studies on confirmed dengue patients hospitalized in Manila. Most of these patients developed mild hemorrhagic manifestations, but the occurrence of shock, particularly severe shock, is uncommon. In two studies conducted during the 1966 epidemic, less than 5% of 321 confirmed dengue cases developed shock.^{4,5} During the 1983-84 study at San Lazaro Hospital, only 7 (4.2%) of 165 DHF cases developed grade III shock, and no case of grade IV shock was seen.⁶ Likewise, in a 1985-86 study, 9 (11%) of 77 DHF patients developed grade III shock, and no grade IV shock was diagnosed.

The occurrence of shock apparently is more common in Thailand and Indonesia where $\geq 30\%$ of the DHF patients have been reported to develop shock.^{14,15} In these areas, shock either preceding or following massive gastrointestinal bleeding has been identified as the major cause of death, and case fatality rates as high as 10–40% have been reported for shock cases.¹⁴ In our study the case fatality rate was only 0.5%. The fatal outcome in both of our patients was attributable to severe shock, but they were not classified as grade IV dengue shock syndrome because concurrent thrombocytopenia and hemoconcentration were not demonstrated. In the other four Philippine studies referred to above, there were no fatalities reported among a total of 996 hospitalized dengue patients.⁴⁻⁷ A substantial number of fatal cases of hemorrhagic fever have been reported during past dengue epidemics in the Philippines,^{3,16} and fatal cases are recorded annually in the national health statistics reports.¹⁷ Unfortunately, most of these cases have not been confirmed serologically or virologically.

Thrombocytopenia has not been a common finding in some past studies on dengue infections in Manila,¹⁸ while other studies have reported platelet counts $< 100,000/\text{mm}^3$ in over 50% of hospitalized dengue patients.⁶⁻⁷ In our

study, thrombocytopenia was found at a significantly higher rate in patients whose platelets were counted by the Unopette® technique than in patients whose platelets were counted by the direct method even though the same technician performed all of the counts. These results suggest that the method used to count platelets is important, however, a comparison using the two methods on the same patients needs to be done to confirm this observation.

In Thailand, DSS has been associated with DEN 2 virus causing secondary infections, whereas in Indonesia, DEN 3 virus has been isolated most frequently from fatal cases.^{14,15} Among the small number of strains isolated in our study, DEN 1 appeared to be associated more frequently with GI bleeding and shock, however, DEN 3 was isolated from one of the fatal cases. In a recent report on dengue isolations from hospitalized patients in the Philippines, DEN 1 also appeared to be associated with more severe disease compared to serotypes 2 and 3.⁷ In another 1983 study on hospitalized dengue patients in Manila, however, DEN 2 was isolated more frequently from patients with gastrointestinal bleeding compared to the other 3 serotypes (Hayes, personal communication).

Most of our patients experienced a 2° type of antibody response. This is in agreement with other recent reports on hospitalized dengue patients in the Philippines.^{6,7} Secondary infections also have been associated with more severe disease in Thailand, especially dengue shock syndrome.¹⁹ In a series of 542 patients with 2° dengue and 71 with 1° dengue, 190 of the former and only 6 of the latter patients developed shock. Based on these observations, Halstead proposed that DHF is due to a self destructive host response; that some persons are sensitized by their first dengue infection. In such a host, the course of a second infection with a virus of a different serotype may be altered adversely by the immune response.

This study shows that dengue infections continue to be a significant cause of pediatric hospitalization in Manila during periods of epidemic activity. Fortunately, most of the current hospitalized cases are mild resulting in a very low case fatality rate from shock or massive hemorrhage. Severe dengue disease apparently is more common in some other large urban areas of Asia, particularly Jakarta and Bangkok, and also may have been more common during some of the earliest outbreaks in Manila. The reasons for such apparent temporal and geographical differences in disease presentation remain to be defined, and physicians should remain alert to the possibility of severe disease associated with future epidemics in Manila.

SUMMARY

Between October 31, 1983 and March 31, 1984, 379 dengue cases were confirmed at the Hospital of Infant Jesus. Most (77.6%) of the patients were 10 years and

below, but only 2 cases were infants. Twenty-six (6.8%) showed a primary antibody response while 322 (84.9%) had a secondary antibody response. Three serotypes of dengue virus were recovered: 13 DEN 3, 12 DEN 1 and 6 DEN 2 strains.

Anorexia, flushing, vomiting, hepatomegaly and abdominal pain were the most frequent nonspecific findings. The most common hemorrhagic manifestations were a positive tourniquet test, petechiae, and epistaxis. Shock was recorded in 33 cases, two of which were fatal. Correlation of antibody response to severity of disease showed that petechiae and GI bleeding were more frequent in cases with a secondary type of response. All cases of shock that could be classified serologically had a secondary antibody response.

This study shows that dengue infection remains a common cause of hospitalization among children in Manila. Hospitalization is apparently more commonly associated with secondary infection.

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